BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

1998 MURRAY

Threonine and Methionine Are Limiting Amino Acids for Protein Synthesis in Patients with AIDS[1,2,3]

Henri Laurichesse, Igor Tauveron,* Florence Gourdon, Louis Cormerais, Claude Champredon,**[4] Sophie Charrier,** Cécile Rochon,** Sylvie Lamain, Gérard Bayle,** Henri Laveran,† Philippe Thieblot,* Jean Beytout and Jean Grizard**

*Service des Maladies Infectieuses et Tropicales, †Service d'Hygiène Hospitalière et *Service d'Endocrinologie et Maladies Métaboliques CHU de Clermont-Ferrand, 63003 Clermont-Ferrand and **Unité d'Etude du Métabolisme Azoté, INRA Centre de Clermont-Ferrand-Theix, 63122 Ceyrat, France*

ABSTRACT This study was conducted to identify the most rate-limiting amino acids for whole-body protein synthesis in acquired immunodeficiency syndrome (AIDS) patients. We postulated that an essential amino acid that would be rate limiting in AIDS should have a low basal plasma concentration and should remain at a low level during amino acid infusion. Seven male AIDS patients (median age 37 y, CD4 cell count: 76 mm^{-3}) without any clinically active opportunistic infection during the month before the experiment were infused intravenously with a complete amino acid-glucose mixture for 2.5 h. Eight healthy volunteers were used as controls. Before the infusion, the concentrations of most free essential amino acids (methionine, threonine, histidine, isoleucine, leucine and tryptophan) were significantly lower ($P < 0.05$) in AIDS patients than in controls. Most plasma free essential amino acids increased significantly during infusion. However, the absolute increase above basal levels for threonine, valine, lysine, ($P < 0.05$) and methionine ($P < 0.073$) was smaller in AIDS patients than in control subjects. Thus, threonine and possibly methionine may be rate limiting for whole-body protein synthesis in AIDS patients, suggesting that there are selective amino acid requirements in patients with AIDS. J. Nutr. 128: 1342–1348, 1998.

KEY WORDS: • *amino acid requirements* • *protein metabolism* • *AIDS* • *limiting amino acids* • *humans*

Infection with the human immunodeficiency virus (HIV)[5] has a devasting effect on nutritional status. Weight loss, often profound in magnitude, is one of the most universal features of HIV infection, and patients may lose 30–50% of their body mass before succumbing to the disease (Gorbach et al. 1993, Sauerwein 1993). Acquired immunodeficiency syndrome (AIDS) is characterized by a predominant loss of lean tissue (Kotler 1985). Multiple factors in different combinations contribute to AIDS related malnutrition and increased host requirements; these include anorexia, malabsorption, abnormal utilization and excretion of nutrients. This is correlated with the severity of the HIV infection and with secondary infections. Malnutrition has a deleterious effect on immune function and thus may potentially accelerate the progression of immune deficiency in HIV infection (Chandra 1991).

Understanding the changes in protein and amino acid metabolism in HIV infection is crucial because they underlie the loss of protein (i.e., of lean tissue). Measurements of amino acid kinetics in AIDS patients have shown the characteristic features of a hypermetabolic response with increased protein turnover (Lieberman et al. 1994, Macallan et al. 1995, Stein et al. 1990). In addition, the degradation of tryptophan via the kynurenine pathway is stimulated (Werner et al. 1988), and sulfur amino acids and glutathione metabolism are altered (Buhl et al. 1989, Eck et al. 1989, Hortin et al. 1994).

Undernutrition may contribute to the protein wasting in HIV patients because nutritional support seems to have been beneficial (Boulétreau et al. 1995, Melchior et al. 1996, Sukkar & Giacosa 1995). Short-term parenteral hyperalimentation enriched with amino acids is capable of reversing net protein catabolism (Macallan et al. 1995, Selberg et al. 1995). However, the amino acid requirements for the replenishment of protein mass in adult humans are not known with certainty (Pellet 1990, Reeds et al. 1994). The aim of this study was

[1] Presented in part in abstract form at the first International Meeting on Nutrition-HIV Infection, April 28–29, 1995, Cannes, France [Charrier, S., Laurichesse, H., Tauveron, I., Laveran, H., Thiéblot, Ph., Grizard, J., Beytout, J. & Champredon, C. (1995) Effect of amino acid infusion on plasma tryptophan concentrations in HIV-infected patients. Preliminary results. Abstract book 103], at the fifth European Conference on Clinical Aspects and Treatment of HIV Infection, September 26–29, 1995, Copenhagen, Denmark. [Laurichesse, H., Tauveron, I., Gourdon, F., Cormerais, L., Charrier, S., Grizard, J., Thiéblot, Ph., Beytout, J. & Champredon, C. (1995) Is threonine a rate limiting amino acid for protein synthesis in AIDS patients? Abstract book 173] and at the XI International Conference on AIDS, July 7–12, 1996, Vancouver, Canada [Champredon, C., Laurichesse, H., Tauveron, I., Gourdon, F., Charrier, S., Cormerais, L., Thiéblot, Ph., Grizard, J. & Beytout, J. (1996) Rate limiting amino acids for protein synthesis in AIDS. Abstract book B 3260].

[2] Supported by a grant from the Agence Nationale de Recherche sur le SIDA, the Direction de la Recherche et des Etudes Doctorales and the Région Auvergne.

[3] The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

[4] To whom correspondence should be addressed.

[5] Abbreviations used: AIDS, acquired immunodeficiency syndrome; BW, body weight: HIV, human immunodeficiency virus.

Manuscript received 14 July 1997. Initial review completed 13 October 1997. Revision accepted 13 April 1998.

EXHIBIT B

12

TABLE 1

Characteristics of the acquired immunodeficiency syndrome (AIDS) patients and the control subjects

	Age, *y*	Height, *m*	Weight, *kg*	BMI, *kg/m²*	CD4+, *mm⁻³*	AIDS-defining events	Current treatment
AIDS patients							
1	30	1.75	48.5	15.8	8	FE, VZ	DDI
2	35	1.60	67	26.2	54	TB	ZVD, DDI, T-SMZ
3	40	1.80	71	21.9	80	PCP	DDC, PA, BT
4	43	1.70	54	18.7	2	Encephalopathy	ZVD, T-SMZ, FC, DHPG, ZC, AC, PZ, FA
5	37	1.84	71	21.0	7	PCP	ZVD, T-SMZ
6	40	1.70	61	21.1	85	TB	ZVD, PA
7	36	1.76	64	20.7	295	KS	ZVD, DDI, T-SMZ, FD
Mean ± SEM[1]	37 ± 2	1.74 ± 0.03	62 ± 3	20.8 ± 1.2	76 ± 39		
Controls							
Mean ± SEM	24 ± 1	1.78 ± 0.01	69 ± 2	21.6 ± 0.5			

[1] Values are means ± SEM, n = 8.

Abbreviations: BMI, body mass index; FE, fungal esophagitis; VZ, herpes zoster; TB, lung tuberculosis; PCP, pneumocystis carinii pneumonia; KS, Kaposi sarcoma; DDI, Didanosine; ZVD, Zidovudine; T-SMZ, Sulfamethoxazole and Trimethoprim; DDC, Dideoxycytidine; PA, Pentamidine aerosol; BT, Betamethasone tablets; FC, Fluconazole; DHPG, Gancyclovir; ZC, Zopiclone; AC, Amoxicillin; PZ, Periciazine; FA, folic acid; FD, fusidic acid.

to define the amino acids that are most limiting for protein anabolism in AIDS patients on the basis of plasma free amino acid response (Pion 1973, Tontisirin et al. 1974, Zello et al. 1995) to a short-term intravenous infusion of an amino acid-glucose mixture.

SUBJECTS AND METHODS

Subjects. The study group consisted of seven men aged 30–43 (median 37 y) recruited from the Department of Infectious Diseases at the University Hospital in Clermont-Ferrand. On the bases of the ELISA and Western blot assay, all were HIV seropositive. A clinical history and a physical examination were performed at the time of the study (Table 1). Six patients were classified C3 (lymphocyte count <200) and one C2 (T4 lymphocytes between 200 and 499) according to the criteria of the Centers for Diseases Control and Prevention (1993). Only patients who had been free of any clinically active opportunistic infection for a period of ≥1 mo before participation were included. The patients' body weight loss was ~5% [compared with pre-illness body weight (BW)]. Patients with fever (>37.8°C) or diarrhea (defined as increased frequency or liquidity of stools) were excluded. Eight male volunteers, who were HIV negative and clinically well, served as a control group. All patients gave a written informed consent. The study protocol was approved by the local Ethics Committee (Comité Consultatif pour la Protection des Personnes en Recherche Biomédicale pour la Région Auvergne).

Experimental procedure. All studies were performed in a postabsorptive state (12-h overnight fast). At 0800 h, a sampling catheter (Venflon 2, 20G, Viggo, Helsingborg, Sweden) was inserted into a dorsal vein of the left forearm. Another catheter was placed in a contralateral forearm vein and used for infusions. Each experiment consisted of a 150-min period of continuous infusion of the amino acid mixture Primene 5% (1 mL/(kg·h); Clintec Technologies, Velizy-Villacoublay, France). Infusions were performed using a peristaltic pump (Infusomat Secura, Braun Biotrol, Paris). The nitrogen content of the amino acid mixture was 7.5 g/L and the amino acid concentration (g/L) was as follows: L-isoleucine 3.35, L-leucine 5.00, L-valine 3.80, L-lysine 5.50, L-methionine 1.20, L-phenylalanine 2.10, L-threonine 1.85, L-tryptophan 1.00, L-alanine 4.00, L-arginine 4.20, L-aspartic acid 3.00, L-cysteine hydrochloride 1.23, L-glutamic acid 5.00, glycine 2.00, L-histidine 1.90, L-proline 1.50, L-serine 2.00, L-tyrosine 0.45, L-ornithine 1.13 and taurine 0.30. A glucose solution (100 g/L; Meram, Melun, France) was concomitantly administered at a constant rate [1 mL/(kg·h)] via the same catheter by a separate [illegible] blood samples (20 mL) were obtained before (−15 and −5 min), during (15, 30, 60, 90, 120 and 150 min) and after the infusion (15 and 30 min). Samples were collected in heparinized tubes, centrifuged at 4°C for 6 min at 3000 mg and plasma stored at −20°C for subsequent analysis.

Assays. Plasma tryptophan was determined by a fluorometric procedure after conversion to norharman by heating in acid conditions with formaldehyde and ferric chloride (Tesseraud et al. 1992). Plasma cyst(e)ine was determined directly by spectrophotometry after coloration with the acid ninhydrin reagent (Malloy et al. 1981). Plasma samples were prepared for analysis of other amino acids by mixing 3 mL plasma with 7 mL of 150 g/L trichloracetic acid containing 0.16 mL thiodiglycol (to prevent methionine oxidation) and 0.75 μmol norleucine (as an internal standard). After storage at 0°C for 1 h and centrifugation at 4000 × g for 30 min at 4°C, the supernatant was passed through a 3-mL cation-exchange column (Dowex AG 50WX8, 100–200 mesh; Bio-Rad, Richmond, CA). Amino acids were eluted from the column with 4 mol/L NH_4OH. The eluate was evaporated to dryness under reduced pressure at 50°C and reconstituted with 3 mL of 0.1 mol/L lithium buffer, pH 2.2. The concentrations of individual amino acids were determined by an automated ion-exchange chromatography apparatus (Biotronic LC 3000, Roucaire, Vélizy, France with BTC 2410 resin), utilizing postcolumn ninhydrin derivatization.

The concentration of other plasma substrates was determined by using an automatic enzymatic analyzer (Chem 1, Bayer Diagnostic, Puteaux, France) with the following enzymes: hexokinase and glucose-6-phosphate dehydrogenase for glucose; urease and glutamate dehydrogenase for urea; cholesterol esterase, cholesterol oxidase and peroxidase for cholesterol; and lipase, glycerokinase, pyruvate kinase and lactate dehydrogenase for triglycerides. Plasma insulin was determined with an immunoenzymatic assay kit (Abbott Diagnostics, Rungis, France). Prealbumin, albumin, retinol binding protein, α_1 acid-glycoprotein and C-reactive protein were measured by immunonephelometry (Beckman, Array System 360, Gagny, France).

Statistical analysis. All data are expressed as means ± SEM. A paired *t* test was used to compare basal data with data obtained during infusions. A two-way ANOVA ($\alpha = 0.05$, main effects: HIV infection and glucose-amino acid infusion) was used to compare results in HIV-infected subjects and controls.

RESULTS

Plasma cholesterol, albumin, prealbumin, retinol binding protein, triglycerides, and C-reactive protein did not differ

TABLE 2

Routine biochemical variables of the acquired immunodeficiency syndrome (AIDS) patients and control subjects

	Controls[1]	AIDS[2]
Cholesterol, *mmol/L*	4.13 ± 0.22	4.16 ± 0.49
Triglycerides, *mmol/L*	0.85 ± 0.09	1.76 ± 0.63
Prealbumin, *g/L*	0.32 ± 0.02	0.30 ± 0.03
Albumin, *g/L*	39.0 ± 1.1	34.9 ± 1.8
Retinol binding protein, *mg/L*	42.4 ± 1.9	45.7 ± 2.7
α_1-Acid glycoprotein, *g/L*	0.64 ± 0.06	0.92 ± 0.08*
C-reactive protein, *mg/L*	<1	<1

[1] Values are means ± SEM, $n = 8$.
[2] Values are means ± SEM, $n = 7$. * $P < 0.05$ vs. controls.

between AIDS patients and controls (**Table 2**). The C-reactive protein was used as a marker to establish the lack of opportunistic infection at the time of the study. By contrast and as expected, the α_1 acid glycoprotein was greater ($P < 0.05$) in AIDS patients than in controls.

Basal plasma insulin concentrations (i.e., before the infusions) did not differ in HIV-infected subjects and in controls (**Fig. 1**). Plasma insulin was stable during the 90- to 50-min infusion period (the CV was 6.4 ± 3.4 and 7.6 ± 3.9% in controls and AIDS patients, respectively) and was not different between the two groups. Nevertheless, the infusion of amino acids plus glucose increased the plasma insulin concentration in AIDS patients ($P < 0.05$) but not in controls (Fig. 1).

Basal plasma glucose concentrations (Fig. 1) did not differ between HIV-infected subjects and controls. The concentrations increased ($P < 0.05$) in the two groups during the first 30-min period of infusions and thereafter reached a plateau (the mean cv was 4.7 ± 0.6 and 3.2 ± 0.4% for the controls and the AIDS patients, respectively, during the 30 to 150-min infusion period). The plateau concentration did not differ between nor did the increase in plasma glucose concentrations above basal. Plasma urea concentrations (Fig. 1) did not differ between groups and decreased significantly to the same extent ($P < 0.05$) during the amino acid-glucose infusion.

In the basal state (**Table 3**), the plasma concentrations of free methionine, threonine, histidine, isoleucine, leucine and tryptophan were significantly lower ($P < 0.05$) in AIDS patients than in controls. There was also a significantly lower concentration ($P < 0.05$) of the nonessential amino acids citrulline, glycine and aspartate plus aspargine in HIV patients. All other amino acid concentrations were not significantly different between groups.

The plasma concentrations of most essential free amino acids significantly increased ($P < 0.05$) during the amino acid-glucose infusion in both groups (**Fig. 2**). However, the absolute increase above basal levels (**Fig. 3**) was significantly lower ($P < 0.05$) in the HIV-infected subjects than in controls for threonine, valine and lysine. This also tended to be the case for methionine ($P = 0.073$). Amino acids increased mainly in the first 30 min of infusion and then reached a plateau (mean cv between 1.3 and 6.5% during 90–150 min). For all amino acids, the concentrations returned to the pre-amino acid infusion concentrations 30 min after termination of the infusions (Fig. 2). Phenylalanine concentration significantly increased ($P < 0.05$) during infusion in the controls but not in the HIV-infected subjects (Fig. 3). Tyrosine decreased ($P < 0.05$) similarly in both groups during infusion. A similar pattern was seen for cyst(e)ine in controls but not in AIDS patients ($P < 0.05$ vs. controls).

Nonessential amino acid concentrations during glucose-amino acids infusions also differed for AIDS patients and control subjects (**Fig. 4**). For example, alanine greatly increased and asparate plus asparagine decreased in controls ($P < 0.05$) but did not change in HIV-infected subjects. The absolute increase in glycine above basal was significantly lower ($P < 0.05$) in AIDS patients than in controls. All other nonessential amino acids were either similarly increased (serine, glutamine plus glutamate and ornithine; $P < 0.05$ vs. basal) or unchanged (citrulline and proline) during infusions in both groups.

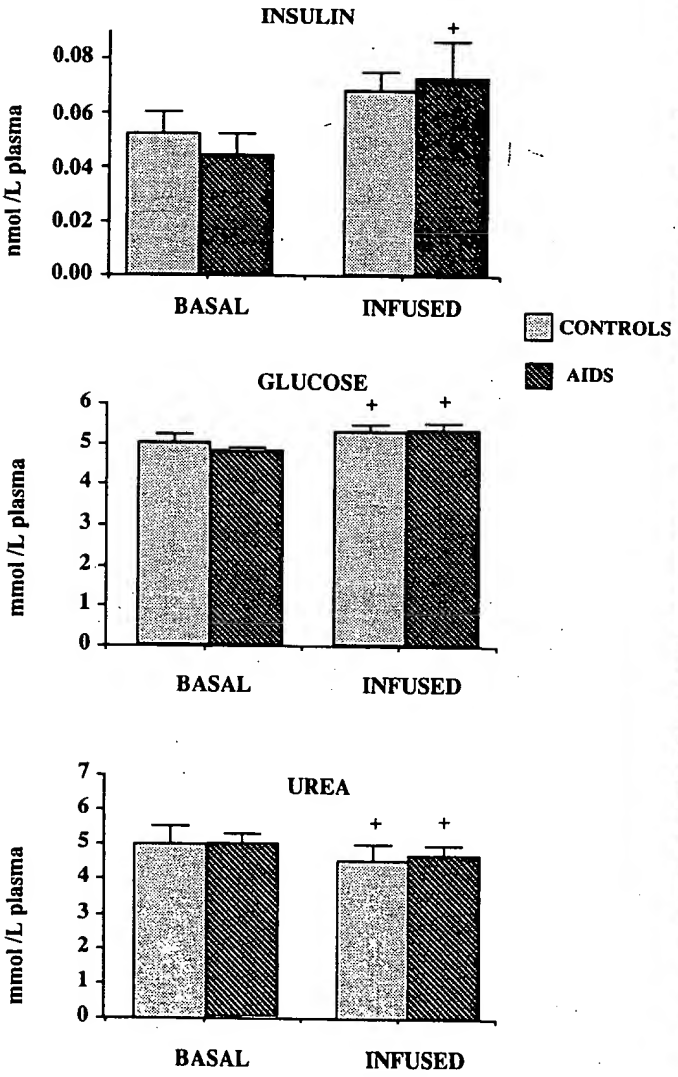

FIGURE 1 Plasma insulin, glucose and urea concentrations in patients with acquired immunodeficiency syndrome (AIDS) and control subjects. All variables were measured before (BASAL) and during the combined amino acid-glucose infusion (INFUSED). Values are means ± SEM for eight controls and seven AIDS patients. The insulin values represent one determination at time −15 min (before infusions, i.e., basal state) and the mean of three determinations at times 90, 120 and 150 min during infusions. The glucose and urea plasma values are the mean of two determinations in the basal state, i.e., times −15 and −5 min (before infusion), and five determinations at times 30, 60, 90, 120 and 150 min during infusions. +INFUSED significantly different from BASAL, $P < 0.05$.

TABLE 3

Basal plasma free amino acids in control subjects and acquired immunodeficiency syndrome (AIDS) patients

	Controls[1]	AIDS[2]	Difference
	μmol/L		%
Essential			
Threonine	123 ± 3	90 ± 9*	−27
Valine	241 ± 9	209 ± 15	−13
Cyst(e)ine	192 ± 8	179 ± 7	−6
Methionine	22 ± 1	16 ± 2*	−30
Isoleucine	63 ± 3	52 ± 4*	−18
Leucine	122 ± 4	101 ± 9*	−17
Tyrosine	57 ± 3	56 ± 4	−2
Phenylalanine	56 ± 1	59 ± 5	+5
Lysine	163 ± 4	163 ± 13	0
Histidine	72 ± 2	59 ± 3*	−18
Arginine	75 ± 3	66 ± 6	−11
Tryptophan	59 ± 2	51 ± 3*	−14
Nonessential			
Aspartate + asparagine	97 ± 3	78 ± 4*	−19
Serine	108 ± 7	95 ± 9	−12
Glutamate + glutamine	422 ± 30	389 ± 54	−8
Glycine	226 ± 10	183 ± 11*	−19
Alanine	324 ± 11	277 ± 27	−14
Citrulline	32 ± 1	22 ± 2*	−31
Ornithine	44 ± 1	48 ± 2	+10
Proline	231 ± 24	165 ± 22	−28

1 Values are means ± SEM, $n = 8$.
2 Values are means ± SEM, $n = 7$. * $P < 0.05$ vs. controls.

DISCUSSION

Loss of lean body mass in HIV patients can result from undernutrition or from disease-induced alterations in metabolism. The lower basal plasma concentrations of most essential ($P < 0.05$ for threonine, methionine, isoleucine, leucine, histidine and tryptophan) and nonessential ($P < 0.05$ only for aspartate plus asparagine, glycine and citrulline) free amino acids in patients with AIDS are consistent with a state of protein undernutrition. Indeed, it has been found that reducing protein supply in rats during constant energy intake results in a decrease in the concentrations of most free amino acids in plasma (Grizard et al. 1977). However, the lower concentrations of plasma free amino acids in HIV patients could not be due to protein deprivation because dietary protein assessment (based on an 8-d diet recall) suggests a more than adequate daily protein intake (2.0 ± 0.3 g/kg BW). In addition, no specific energy restriction was detected (daily intake at 222 ± 29 kJ/kg BW) in these subjects. Our study therefore adds evidence to the hypothesis that a state of undernutrition does not exist in stable HIV patients, defined as those free of clinically active opportunistic infections, fever and diarrhea (Sauerwein 1993). However, these adequate intakes did not preclude muscle wasting, and many HIV patients have a history of long-term weight loss.

The lower concentrations of plasma free amino acids in fasting AIDS patients are presumably more a reflection of a stressed state associated with increased protein turnover and net catabolism. Most plasma free amino acid concentrations are decreased during stress, trauma and sepsis (Sax et al. 1988, Vente et al. 1989). Although HIV infection demonstrates many of the characteristics of a catabolic process, the initial

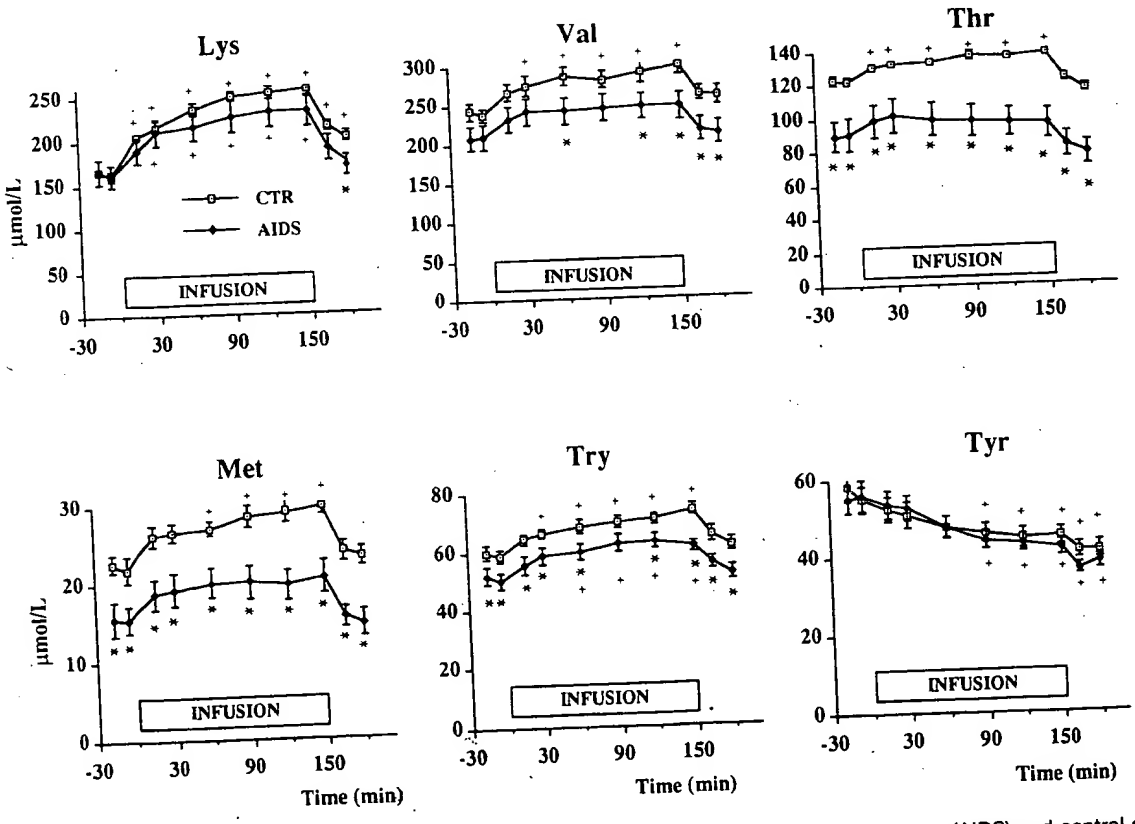

FIGURE 2 Plasma free amino acid concentrations in patients with acquired immunodeficiency syndrome (AIDS) and control subjects. Amino acids were assayed at various times in each group during a basal period (times −15 and −5 min), during the combined amino acid-glucose infusions (indicated by open boxes) and after the infusions (times 165 and 180 min). Values are means ± SEM for eight controls and seven AIDS patients. *Significantly different from controls, $P < 0.05$. ˜Significantly different from the values at −15 or −5 min in the same group, $P < 0.05$.

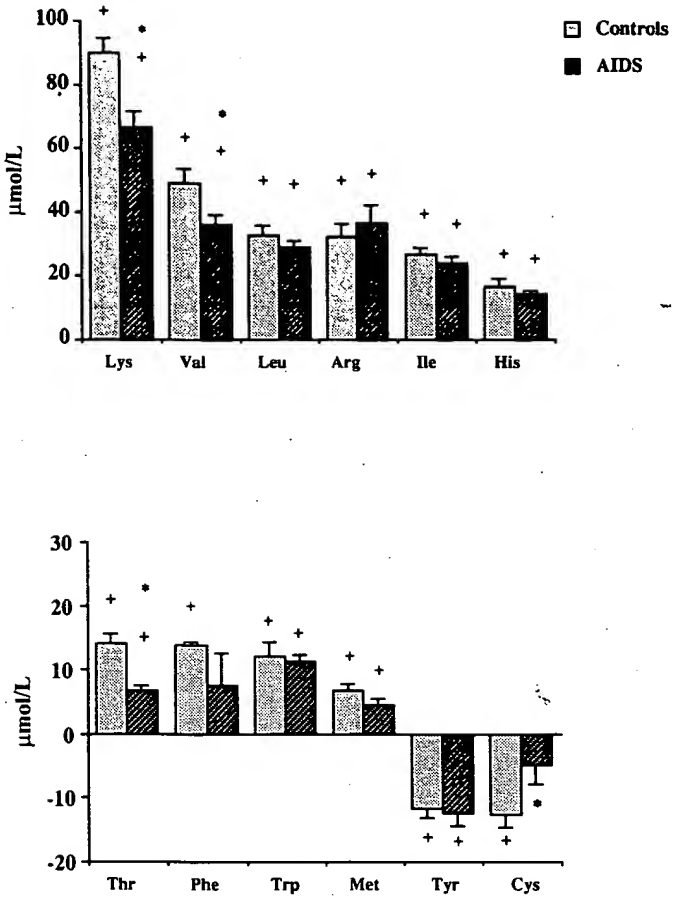

FIGURE 3 The absolute increase in plasma essential free amino acid concentrations during combined amino acid-glucose infusions in control subjects and acquired immunodeficiency syndrome (AIDS) patients. The plasma free amino acids concentrations were measured at times −15 and −5 min (before infusions) and at times 90, 120 and 150 min during infusions. The absolute increase of each amino acid represents the difference between the mean concentration obtained during and before infusions. Values are means ± SEM for eight controls and seven human immunodeficiency virus (HIV)-infected patients. *Significantly different from controls, $P < 0.05$. +Significantly different from zero, $P < 0.05$.

study of protein metabolism in HIV infection using [^{15}N] glycine as a tracer (Stein et al. 1990) found reduced rates of whole-body protein turnover. However, more recent investigations using [^{13}C] leucine have suggested that the rates of protein turnover are high in cachectic AIDS patients (Lieberman et al. 1994, Macallan et al. 1995).

Increased protein turnover and catabolism should increase dietary protein requirements. Our study was therefore conducted to identify possible changes in individual amino acid requirements in patients with AIDS. Dietary amino acid requirements for adult humans have been determined by a number of different methods. Historically, descriptive or gross measurements such as nitrogen balance have been used. However, technological advancements have resulted in the use of more precise and mechanistic metabolic approaches to examine requirements (i.e., plasma amino acid concentrations, amino acid oxidation and indicator amino acid oxidation).

Our approach is based on the fact that when amino acids are provided at insufficient levels (limiting amino acids), most of these amino acids will be used efficiently for protein synthesis, and plasma free concentrations and oxidation will remain low and constant. In contrast, the amino acids in excess of the amounts needed for protein synthesis accumulate and are preferentially oxidized by the body. As the supply of the limiting amino acids increases above the requirements for protein synthesis, increased concentration and catabolism of these amino acids ensue. This method, initially developed in animal experiments, has also been used in human studies (Fuller and Garlick 1994, McLarney et al. 1996, Zello et al. 1995).

On the basis of this concept, limiting amino acids should be able to be identified from plasma free amino acid concentrations in response to a fixed amino acid infusion. During amino acid infusion, the inhibition of protein synthesis and anabolism due to limiting amino acids will be suppressed; as a result, more amino acids will be incorporated into body proteins. The concentrations of nonlimiting amino acids will be altered, depending on the difference between supply by the perfusion and utilization for body deposition and oxidation. The plasma concentrations of limiting amino acids will stay at a low level if their supply from the infusion compensates only for their utilization. These amino acids will accumulate when in excess. In other words, amino acids that have a low basal level and do not change during infusion give indication that they are limiting amino acids for protein anabolism.

Studies investigating the fate of infused amino acids are consistent with these concepts. It has been shown in healthy volunteers that an infusion of mixed amino acids stimulates whole-body leucine disappearance through both oxidative and nonoxidative pathways (Bennet et al. 1989, Castellino et al. 1987 and 1992, Fukagawa et al. 1989, Pacy et al. 1988, Tessari et al. 1987). An even greater inhibition of endogenous leucine appearance was also seen when amino acids were combined with glucose and insulin (Bennet et al. 1990, Castellino et al. 1987, Flakoll et al. 1989, Fukagawa et al. 1989, Heslin et al. 1992, Tauveron et al. 1995, Tessari et al. 1987). The splanchnic bed is the major site of the disposal of intravenously administrated amino acids (Gelfand et al. 1986). Studies in animals suggest that intravenous amino acids are catabolized preferentially by the liver and thus reduce the amounts of amino acids arising from proteolysis (Mortimore et al. 1987). Liver protein

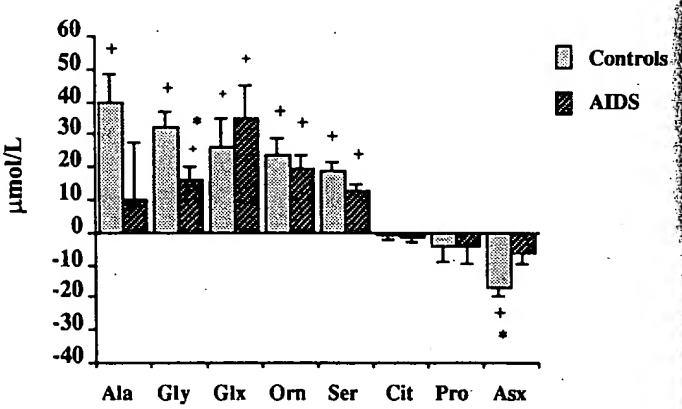

FIGURE 4 The absolute increase of plasma nonessential free amino acid concentrations during combined amino acid-glucose infusions in control subjects and acquired immunodeficiency syndrome (AIDS) patients. The plasma free amino acid concentrations were measured at times −15 and −5 min (before infusions) and at times 90, 120 and 150 min during infusions. The absolute increase of each amino acid represents the difference between the mean concentration obtained during and before infusions. Values are means ± SEM for eight controls and seven AIDS patients. *Significantly different from controls, $P < 0.05$. +Significantly different from zero, $P < 0.05$. Glx = Glu + Gln. Asx = Asp + Asn.

synthesis may also be stimulated (Tauveron et al. 1994). Amino acid deposition in skeletal muscle cannot be ruled out because an increase in muscle protein synthesis by hyperaminoacidemia has been reported in both healthy volunteers (Bennet et al. 1989) and animals (Mosoni et al. 1993, Watt et al. 1992). Interestingly, it has been shown that the acute anabolic response to intravenous amino acid infusion was normal in HIV-infected subjects (Macallan et al. 1995, Selberg et al. 1995).

The magnitude of change in most essential plasma free amino acid concentrations during infusion in this study (an increase in leucine, arginine, isoleucine, histidine and tryptophan and a decrease in tyrosine) was similar in controls and HIV-infected subjects. Because the infusion rate was the same in the two groups, the changes in protein metabolism were roughly the same in the two groups. However, the increase in some other essential amino acids was smaller in HIV patients (lysine, valine and threonine) than in control subjects. This could be a reflection of increased basal protein turnover and amino acid oxidation.

Of the limiting amino acids, only the essential amino acids threonine and perhaps methionine met our criteria (see above). For example, the basal level of threonine was among the most depressed of the amino acids in HIV-infected subjects (−27% compared with controls). In addition, the absolute increase in plasma free threonine after infusions (although significant, $P < 0.05$) was very modest in HIV-infected subjects, representing only 52% of the increase in controls. Based on the curve describing blood free threonine in response to the consumption of graded levels of threonine (Pion 1973, Tontisirin et al. 1974), our results suggest that HIV-infected patients have a selective deficiency in threonine. Such a deficiency has also been demonstrated recently in septic rats by using amino acid balance methodologies (Arnal et al. 1995). This selective threonine deficiency could arise from an activation of the catabolism of threonine and/or synthesis of threonine-rich proteins.

A minor change in methionine was also noted after infusion, along with low basal levels in HIV-infected subjects. This amino acid deficiency is consistent with the known alterations in sulfur amino acid metabolism that occur in AIDS patients. An activation of the metabolism of cyst(e)ine, especially to taurine, may occur in HIV-infected patients (Hortin et al. 1994). To explain the decrease in plasma free methionine, we hypothesize that in patients with AIDS, there is a concomitant activation of the metabolism of methionine to cyst(e)ine. In contrast to previous studies (Hortin et al. 1994), cyst(e)ine depletion was not observed in our experiments although abnormal kinetics were recorded during infusions. We also hypothesize that more sulfur amino acids are needed in patients with AIDS to meet their requirements for glutathione (γ-glutamylcysteinylglycine) synthesis. Cysteine is both a precursor and a regulator of glutathione synthesis. HIV-infected patients are glutathione deficient (López Galera et al. 1996), presumably as a result of an enhanced utilization due to activation of lymphocytes and cell-mediated cytotoxic function and protection against oxidative damage. Similar mechanisms have been proposed as explanations for the increased sulfur amino acid requirements during sepsis in rats (Malmezat et al. 1998).

Previous studies indicated that the degradation of tryptophan via the kynurenine pathway is stimulated in HIV-infected subjects (Werner et al. 1988). This may contribute to the neurologic symptoms often associated with the HIV infection. Although the basal plasma tryptophan concentrations were significantly lower in HIV patients than in controls, this amino acid increased similarly after infusion in both groups in our experiment and thus could not be considered rate limiting as was the case for threonine and methionine.

It is noteworthy that the nonessential amino acid glycine, exhibited the same behavior as a limiting amino acid. Glycine is metabolically related to serine, methionine, cysteine and threonine. These related amino acids are very abundant in many proteins synthesized in increased amounts during infection, trauma and chronic inflammatory diseases (see Grimble 1990 for a review). In this study, the utilization of glycine for glutathione synthesis was also presumably enhanced (see above). Alternatively, the appearance rate of glycine may have been decreased, first, because glycine synthesis from serine may have been decreased as a result of an increased utilization of serine for cysteine synthesis and second, because glycine can also be synthesized from threonine. We speculate that changes in threonine metabolism led to a decrease in glycine appearance. A drastic decrease in plasma free glycine has been observed in patients in response to multiple traumas (Grimble 1990).

The postabsorptive concentrations of free amino acids in plasma from HIV-infected subjects are more consistent with a septic situation rather than a state of protein deprivation or energy restriction. By using acute amino acid plus glucose infusions, we were able to detect selective amino acid deficiencies, especially with threonine and methionine. Methionine depletion correlated with the known alterations in sulfur amino acid metabolism during AIDS. In contrast, threonine depletion is a new concept that should be taken into account in AIDS nutrition. Further studies are needed to elucidate the changes in threonine metabolism and to determine whether this amino acid contributes to the pathophysiology of HIV infection.

ACKNOWLEDGMENTS

The authors thank Clintec Technologies for providing Primene 5% and Anthony Kee for improving the manuscript.

LITERATURE CITED

Arnal, M., Rosé, G., Breuillé, D. & Obled C. (1995) Composition à base d'acides aminés destinée au traitement du sepsis ou d'une agression engendrant une réaction inflammatoire chez les animaux et chez l'homme. Brevet n° 931288K publié en France.

Bennet, W. M., Connacher, A. A., Scrimgeour, C. M., Jung, R. T. & Rennie, M. J. (1990) Euglycemic hyperinsulinemia augments amino acid uptake by human leg tissues during hyperaminoacidemia. Am. J. Physiol. 259: E185–E194.

Bennet, W. M., Connacher, A. A., Scrimgeour, C. M., Smith, K. & Rennie, M. J. (1989) Increase in anterior tibialis muscle protein synthesis in healthy man during mixed amino acid infusion: studies of incorporation of [1-^{13}C] leucine. Clin. Sci. (Lond.) 76: 447–454.

Boulétreau, P., Gérard, M., Messing, B., Chambrier, C., Gelas, P., Robert, D., Bryssine, S. & Khalfallah, S. (1995) Home parenteral nutrition and AIDS. Clin. Nutr. 14: 213–218.

Buhl, R., Jaffe, H. A., Holroyd, K. J., Wells, F. B., Mastrangeli, A., Saltini, C., Cantin, A. M. & Crystal, R. G. (1989) Systemic glutathione-deficiency in symptom free HIV seropositive individuals. Lancet 2: 1294–1297.

Castellino, P., Luzi, L., Simonson, D. C., Haymond, M. & DeFronzo, R. A. (1987) Effect of insulin and plasma amino acid concentrations on leucine metabolism in man. Role of substrate availability on estimates of whole body protein synthesis. J. Clin. Invest. 80: 1784–1793.

Castellino, P., Solini, A., Luzi, L., Barr, J. G., Smith, D. J., Petrides, A., Giordano, M., Carroll, C. & DeFronzo, R. A. (1992) Glucose and amino acid metabolism in chronic renal failure—effect of insulin and amino acids. Am. J. Physiol. 262: F168–F176.

Centers for Disease Control and Prevention (1993) Revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. Morb. Mortal. Wkly. Rep. 41: 1–19.

Chandra, R. K. (1991) Nutrition and immunity: lessons from the past and new insights into the future. Am. J. Clin. Nutr. 53: 1087–1101.

Eck, H. P., Gmünder, H., Hartmann, M., Petzoldt, D., Daniel, V. & Dröge, W. (1989) Low concentrations of acid-soluble thiol (cysteine) in the blood plasma of HIV-1-infected patients. Biol. Chem. Hoppe-Seyler 370: 101–108.

Flakoll, P. J., Kulaylat, M., Frexes-Steed, M., Hourani, H., Brown, L. L., Hill, J. O. &

Abumrad, N. N. (1989) Amino acids augment insulin's suppression of whole body proteolysis. Am. J. Physiol. 257: E839–E847.
Fukagawa, N. K., Minaker, K. L., Young, V. R., Matthews, D. E., Bier, D. M., & Rowe, J. W. (1989) Leucine metabolism in aging humans: effect of insulin and substrate availability. Am. J. Physiol. 256: E288–E294.
Fuller, M. F. & Garlick, P. J. (1994) Human amino acid requirements: can the controversy be resolved? Annu. Rev. Nutr. 14: 217–241.
Gelfand, R. A., Glickman, M. G., Jacob, R., Sherwing, R. S. & DeFronzo, R. A. (1986) Removal of infused amino acids by splanchnic and leg tissues in humans. Am. J. Physiol. 250: E407–E413.
Gorbach, S. L., Knox, T. A. & Roubenoff, R. (1993) Interactions between nutrition and infection with human immunodeficiency virus. Nutr. Rev. 51: 226–234.
Grimble, R. F. (1990) Nutrition and cytokine action. Nutr. Res. Rev. 3: 193–210.
Grizard, J., Prugnaud, J. & Pion, R. (1977) Influence d'un excés d'insuline sur la composition corporelle et les teneurs en acides aminés libres du sang, du foie et du muscle du rat en croissance soumis ou non à une restriction azotée. Ann. Biol. Anim. Biochim. Biophys. 17: 373–387.
Heslin, M. J., Newman, E., Wolf, R. F., Pisters, P.W.T. & Brennan, M. F. (1992) Effect of hyperinsulinemia on whole body and skeletal muscle leucine carbon kinetics in humans. Am. J. Physiol. 262: E911–E918.
Hortin, G. L., Landt, M. & Powderly, W. G. (1994) Changes in plasma amino acid concentrations in response to HIV-1. Clin. Chem. 40: 785–789.
Kotler, D. P., Wang, J. & Pierson, R. N. (1985) Body composition studies in patients with the acquired immunodeficiency syndrome. Am. J. Clin. Nutr. 42: 1255–1265.
Lieberman, S. A., Butterfield, G. E., Harrison, D. & Hoffman, A. R. (1994) Anabolic effects of recombinant insulin-like growth factor-I in cachectic patients with the acquired immunodeficiency syndrome. J. Clin. Endocrinol. Metab. 78: 404–410.
López Galera, R. M., Juárez Giménez, J. C., Montoro Ronsano, J. B., Segura Cardona, R. M., Arbós Via, M. A., Altisent Roca, C. & Tusell Puigbert, J. M. (1996) Glutathione and cysteine in HIV-infected hemophiliacs. Clin. Chim. Acta 254: 63–72.
Macallan, D. C., McNurlan, M. A., Milne, E., Calder, A. G., Garlick, P. J. & Griffin, G. E. (1995) Whole-body protein turnover from leucine kinetics and the response to nutrition in human immunodeficiency virus infection. Am. J. Clin. Nutr. 61: 818–826.
Malloy, M. H., Rassin, D. K. & Gaull, G. E. (1981) A method for measurement of free and bound plasma cyst(e)ine. Anal. Biochem. 113: 407–415.
Malmezat, T., Breuillé, D., Pouyet, C., Patureau Mirand, P. & Obled, C. (1998) Metabolism of cysteine is modified during the acute phase of sepsis in rats. J. Nutr. 128: 97–105.
McLarney, M. J., Pellett, P. L. & Young, V. R. (1996) Pattern of amino acid requirements in humans: an interspecies comparison using published amino acid requirements recommendations. J. Nutr. 126: 1871–1882.
Melchior, J. C., Chastang, C., Gelas, P., Carbonnel, F., Zazzo, J. F., Boulier, A., Cosnes, J., Boulétreau, P. & Messing, B. (1996) Efficacy of 2-month total parenteral nutrition in AIDS patients: a controlled randomized prospective trial. AIDS 10: 379–384.
Mortimore, G. E., Pösö, A. R., Kadowaki, M. & Wert, J. J. (1987) Multiphasic control of hepatic protein degradation by regulatory amino acids. General features and hormonal modulation. J. Biol. Chem. 262: 16322–16327.
Mosoni, L., Houlier, M. L., Patureau Mirand, P., Bayle, G. & Grizard, J. (1993) Effect of amino acids alone or with insulin on muscle and liver protein synthesis in adult and old rats. Am. J. Physiol. 264: E614–E620.
Pacy, P. J., Garrow, J. S., Ford, G. C., Merritt, H. & Halliday, D. (1988) Influence of amino acid administration on whole-body leucine kinetics and resting metabolic rate in postabsorptive normal subjects. Clin. Sci. (Lond.) 75: 225–231.
Pellett, P. L. (1990) Protein requirements in humans. Am. J. Clin. Nutr. 51: 723–737.
Pion, R. (1973) The relationships between the levels of free amino acids in blood and muscle and the nutritive value of proteins. In: Protein in Human Nutrition (Porter, J. W. & Rolls, B. A., eds.), pp. 329–341. Academic Press, London, UK.
Reeds, P. J., Fjeld, C. R. & Jahoor, F. (1994) Do the differences between the amino acid compositions of acute-phase and muscle proteins have a bearing on nitrogen loss in traumatic states? J. Nutr. 124: 906–910.
Sauerwein, H. P. (1993) Nutrition and AIDS. Clin. Nutr. 12: S64–S68.
Sax, H. C., Hasselgren, P.-O., Talamini, M. A., Edwards, L. L. & Fischer, J. E. (1988) Amino acid uptake in isolated, perfused liver: effect of trauma and sepsis. J. Surg. Res. 45: 50–55.
Selberg, O., Süttmann, U., Melzer, A., Deicher, H., Müller, M. J., Henkel, E. & McMillan, D. C. (1995) Effect of increased protein intake and nutritional status on whole-body protein metabolism of AIDS patients with weight loss. Metabolism 44: 1159–1165.
Stein, T. P., Nutinsky, C., Condoluci, D., Schluter, M. D. & Leskiw, M. J. (1990) Protein and energy substrate metabolism in AIDS patients. Metabolism 39: 876–881.
Sukkar, S. G. & Giacosa, A. (1995) Home nutritional support in AIDS patients. Clin. Nutr. 14: 41–45.
Tauveron, I., Charrier, S., Champredon, C., Bonnet, Y., Berry, C., Bayle, G., Prugnaud, J., Obled, C., Grizard, J. & Thiéblot, P. (1995) Response of leucine metabolism to hyperinsulinemia under amino acid replacement in experimental hyperthyroidism. Am. J. Physiol. 269: E499–E507.
Tauveron, I., Larbaud, D., Champredon, C., Debras, E., Tesseraud, S., Bayle, G., Bonnet, Y., Thiéblot, P. & Grizard, J. (1994) Effect of hyperinsulinemia and hyperaminoacidemia on muscle and liver protein synthesis in lactating goats. Am. J. Physiol. 267: E877–E885.
Tessari, P., Inchiostro, S., Biolo, G., Trevisan, R., Fantin, G., Marescotti, M. C., Iori, E., Tiengo, A. & Crepaldi, G. (1987) Differential effects of hyperinsulinemia and hyperaminoacidemia on leucine-carbon metabolism in vivo. J. Clin. Invest. 79: 1062–1069.
Tesseraud, S., Grizard, J., Makarski, B., Debras, E., Bayle, G. & Champredon, C. (1992) Effect of insulin in conjunction with glucose, amino acids and potassium on net metabolism of glucose and amino acids in the goat mammary gland. J. Dairy Res. 59: 135–149.
Tontisirin, K., Young, V. R., Rand, W. M. & Scrimshaw, N. S. (1974) Plasma threonine response curve and threonine requirements in young men and elderly women. J. Nutr. 104: 495–505.
Vente, J. P., Von Meyenfeldt, M. F., Van Eijk, H.M.H., Van Berlo, C.L.H., Gouma, D. J., Van der Linden, C. J. & Soeters, P. B. (1989) Plasma-amino acid profiles in sepsis and stress. Ann. Surg. 209: 57–62.
Watt, P. W., Corbett, M. E. & Rennie, M. J. (1992) Stimulation of protein synthesis in pig skeletal muscle by infusion of amino acids during constant insulin availability. Am. J. Physiol. 263: E453–E460.
Werner, E. R., Fuchs, D., Hausen, A., Jaeger, H., Reibnegger, G., Werner-Felmayer, G., Dierich, M. P. & Wachter, H. (1988) Tryptophan degradation in patients infected by human immunodeficiency virus. Biol. Chem. Hoppe-Seyler 369: 337–340.
Zello, G. A., Wykes, L. J., Ball, R. O. & Pencharz, P. B. (1995) Recent advances in methods of assessing dietary amino acid requirements for adult humans. J. Nutr. 125: 2907–2915.